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(54) Title: ORAL DELIVERY OF PEPTIDES (57) Abstract <p>The present invention provides a pharmaceutical composition for the delivery of proteins and/or polypeptides, comprising: (i) one or more therapeutically effective peptides or polypeptides; and (ii) a chelating agent, wherein said composition is adapted to pre-deliver the chelating agent. Also described is a method of enhancing the absorption of therapeutically effective, biologically active polypeptides or proteins so as to achieve a physiological effect in a patient requiring such therapy, wherein the method comprises the step of: (i) administering to said patient in an oral formulation, an effective amount of a protein or polypeptide, and a chelating agent, said chelating agent being pre-delivered with respect to said protein or polypeptide.</p>		

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ORAL DELIVERY OF PEPTIDES

This invention relates to the pharmaceutical delivery of biologically active peptides, and or polypeptides by oral administration, and thence by the intestinal route, to achieve a therapeutic physiological effect.

- 5 Oral administration of pharmaceuticals provides many advantages that alternative routes of delivery do not. For example, oral administered drugs are simple for a patient to self administer obviating the requirement for frequent professional medical intervention in chronic disorders. Proteins and polypeptide sequences have however proven problematic to deliver because, under normal
- 10 physiological conditions in the mammal, proteins are hydrolyzed by digestive enzymes to small peptides and free amino acids, preventing the absorption of the intact biologically active form of the protein. Furthermore, under usual physiological circumstances most ingested proteins are not absorbed intact from the gastrointestinal tract, being too large in molecular mass to cross the
- 15 intestinal epithelium as intact molecules.

There are reports that some proteins are absorbed intact across the gastrointestinal mucosal barrier in amounts up to 2% of the administered dose. It is postulated that these particular proteins may contain structural features permitting their absorption across the gastrointestinal tract as intact molecules.

- 20 Under rigorous testing conditions however, the absorption across mucosal barriers of peptide and polypeptide sequences has not proven practical with the exception of β -lactam antibiotics and the small peptide-like inhibitors of angiotensin converting enzyme. (Saito, H., et al. (1993) "Expression of human intestinal dipeptide transporter in *Xenopus-Laevis* oocytes." Biochemical
- 25 Pharmacology, 45, 776-779; Kramer, W. et al. (1992) "Intestinal absorption of beta-lactam antibiotics and oligopeptides. Functional and stereospecific reconstitution of the oligopeptide transport system from rabbit small intestine." Eur J Biochem, 204, 923-930; Kramer, W. et al. (1990) "Intestinal absorption of dipeptides and beta-lactam antibiotics .II. Purification of the binding protein for

- dipeptides and beta-lactam antibiotics from rabbit small intestinal brush border membranes." Biochim Biophys Acta, 1030, 50-59; Kramer, W., et al. (1990) "Intestinal uptake of dipeptides and beta-lactam antibiotics. I. The intestinal uptake system for dipeptides and beta-lactam antibiotics is not part of a brush border membrane peptidase." Biochim Biophys Acta, 1030, 41-49). Several reports have described the increased absorption of intact proteins particularly during the administration of inhibitors of protein digestion and hydrolysis such as Trasylol or aprotinin. (Kararli, T.T., et al., (1992) "Oral delivery of a renin inhibitor compound using emulsion formulations." Pharm Res, 9, 888-893; Takada, K., et al., (1994) "Pharmacological activity of tablets containing recombinant human granulocyte colony stimulating factor (rhG-CSF) in rats." Int J Pharm 101, 89-96; Bendayan, M., et al. (1994) "Biochemical and morpho-cytochemical evidence for the intestinal absorption of insulin in control and diabetic rats - comparison between the effectiveness of duodenal and colon mucosa." Diabetologia, 37, 119-126; Zhou, X.H., (1994) "Overcoming enzymatic and absorption barriers to non-parentally administered protein and peptide drugs." J. Control Release, 29, 239-252) It is clear however, that in these cases the passage of the protein or polypeptide is assisted by the anti-enzymic effect of the additives to the formulation.
- Bile salts have also been shown to increase the absorption of proteins such as insulin. However at the high concentrations needed to achieve significant absorption of the proteins, the bile salts are toxic to the cells lining the wall of the intestine, and hence this solution is not practicably feasible. A recent variation on this development is the facilitation of absorption of peptides coupled to bile acids. (Kramer, W., et al., (1994) "Intestinal absorption of peptides by coupling to bile acids." J. Biol. Chem., 269 10621-10627).

An alternative mechanism of delivery of peptides so as to achieve enteral absorption is to enhance absorption by co-delivery of surfactants and polymers, examples of which are polyoxyethylene-24-cholesteryl ether (Drewe, J. et al, (1993) "Enteral absorption of octreotide-absorption enhancement by polyoxyethylene-24-cholesteryl ether." British Journal of Pharmacology 108, 298-303); or poly acrylic acid derivatives and chitosans (Luessen, H.L., (1994)

- "Bioadhesive polymers for the peroral delivery of peptide drugs." J. Control. Release 29, 329-338). In a refinement of this approach bioadhesive co-polymers of fumaric acid and sebacic acid in a microsphere formulation increased the intestinal absorption of insulin (Mathiowitz, E., et al (1997)
- 5 "Biologically erodable microspheres as potential oral drug delivery systems. Nature, 386, 410-414).

Other absorption enhancers can be classified as follows:

1. Fatty acids such as capric, oleic and linoleic acids have been found to have strong enhancing properties with no damage to the mucosa;
- 10 2. Surfactants such as sodium lauryl sulfate (SLS) and chelating agents have been studied repeatedly and found to enhance absorption but damage the mucosa; and
- 15 3. Liquid compounds such as dimethylsulfoxide (DMSO) and N,N-dimethylacetamide (DMAC) have been used to enhance absorption of macromolecules but their use has been associated with systemic side effects.

Chelating agents have also been studied for promoting the absorption of drugs and proteins. In particular, EDTA has been used to promote the nasal and

20 intestinal absorption of macromolecules. However, the use of EDTA has been discouraged since damage to the intestinal mucosa has been shown. For example, Nakanishi et al. found that Na₂EDTA (0.8% w/v) caused a reversible loss of rectal epithelial cells (Nakanishi, K., et al (1983) "Effect of pharmaceutical adjuvants on rectal permeability of drugs. III. Effect of repeated administration and recovery of the permeability." Chem. Pharm. Bull., 31, 4161-4166). In other

25 experiments in rats within 90 minutes of incubation of Na₂EDTA (25mM) there was a 30% loss of epithelial cells and goblet cells from the intestinal mucosa (Nakanishi, K., et al. (1982), "Effect of pharmaceutical adjuvants on the rectal

permeability of drugs. I Effect of pharmaceutical adjuvants on permeability of sulfaguanidine from the rat rectum." Yakugakuzasshi 102, 1133-1140). Bleeding was also found to occur after bolus delivery of Na₂EDTA in loops of dog jejunum (Tidball, C.S., and Lipman, R.I. (1962) "Enhancement of jejunal absorption of
5 heparinoid by sodium ethylenediaminetetraacetate in the dog." Proc. Soc. Exp. Biol. Med., 111, 713-715). In another study in rats, Na₂EDTA (0.8%) induced epithelial cell loss and damage to blood capillaries as shown by light microscopy (Nadai, T., et al., (1975), "Drug induced histological changes and its consequences on the permeability of the small intestinal mucosa. I. EDTA,
10 tetracycline and sodium laurylsulfate." Chem. Pharm. Bull., 20, 1139-1140).

In 1989 Van Hoogdalem, E.J. et al. reported that EDTA (0.25%) was ineffective in promoting rectal absorption of a neuroleptic peptide in conscious rats (Van Hoogdalem, E.J. et al. (1989) "Rectal absorption enhancement of Des-Enkephalin-gammaendorphin by medium chain glycerides and EDTA in
15 conscious rats." Pharmaceut. Res., 6, 91-95).

EDTA has also been studied as an absorption enhancer across the mucosal membranes of the rectum, the jejunum, the colon, the nasal and the buccal regions.

Various patents have also been filed in respect of the principle of enhancing
20 peptide delivery across buccal membranes (eg. US 4,476,116) and across membranes in the eye (eg. US 5,283,236).

None of the patent or scientific documents report however, the successful enhancement of intestinal peptide delivery in intact animals. This is largely due to the difficulty in transition from *in vivo* to *in vitro* delivery. Additionally, none of
25 the routes tested are particularly suited to the self- administration of a peptide-or protein-based drugs by a patient without the need for professional medical intervention.

It is an object of this invention to provide an alternative mechanism of oral administration of peptides and or polypeptides which addresses the difficulties confronted by the methods proposed to date. In particular, it is an object of this invention to provide a formulation for oral delivery of proteins and polypeptides, 5 which formulation achieves delivery of effective amounts of proteins or polypeptides across the gastrointestinal mucosal barrier, so as to achieve a therapeutic physiological effect. It is a further object of this invention to provide a method of orally treating a patient requiring protein or polypeptide-based treatment so as to achieve a therapeutic effect.

- 10 Thus, the present invention provides a pharmaceutical composition for the delivery of proteins and/or polypeptides, comprising: (i) one or more therapeutically effective peptides or polypeptides; and (ii) a chelating agent, wherein said composition is adapted to pre-deliver the chelating agent.

This invention is predicated on the discovery that the pre-delivery of a chelating 15 agent in oral peptide delivery formulations results in unexpectedly high gastrointestinal absorption of the peptide of interest, resulting in a potential therapeutic effect. It is believed that the intact absorption of functional peptides and polypeptides may be due to a binding of cations such as calcium by the chelator, and or to the activation of a specific cell transport system.

- 20 Various pathways are believed to be involved in the absorption of peptides and proteins across the intestinal epithelium according to the invention. The first pathway involves the passage of peptides and proteins through the cell membrane (transcellular route). The second pathway involves the movement of peptides and proteins through intercellular spaces between cells (paracellular 25 route). In each instance, chelating agents such as EDTA and EGTA, are thought to chelate extracellular ions from membranes or cellular junctions resulting in a consequential enhancement of peptide and polypeptide absorption. Such chelators may also enhance adsorption by removing ions required by proteolytic enzymes during proteolysis thereby substantially reducing the 30 proteolytic activity of the enzymes.

- The transfer of peptides and proteins across the intestinal mucosa is thought to also involve interactions with macrophages and or lymphocytes, both of which are present in the mucosa or within specialised regions of the intestinal wall. Under normal physiological circumstances, proteins escaping digestion are
- 5 absorbed across the intestinal mucosa, they are then degraded by macrophages in the submucosa, presumably as part of the immunological surveillance system of the body. However, by pretreating the intestinal environment with one or more chelators the activity of the macrophages can be suspended temporarily so that intact functional proteins may be absorbed into the body.
- 10 The efficacy of the above invention in delivering peptides and proteins depends on the predelivery of a chelator, before the delivery and or release of the peptides or proteins. If a chelator is delivered at the same time as a peptide or protein there is little absorption of the peptide or protein across the mucosal membrane. However, a marked difference in peptide or protein absorption
- 15 occurs where there is a delay between release of the chelator and the peptide or protein. Preferably, the formulation of the present invention is adapted to release the chelator upon entry into the intestinal tract or soon thereafter, then after a suitable time period release the peptide or protein. Desirably, the delay between the release of the chelator and the protein or peptide is greater than
- 20 about 30 minutes. Most preferably, the chelator is released 60 to 120 minutes prior to the release of the peptide or protein of interest. In one embodiment of the invention the chelator is pre-delivered at least 2 hours before the peptide or protein.

- Pharmaceutical compositions produced according to the invention may be used
- 25 to any peptide or polypeptide preparation. Desirably the composition is used to deliver peptides such as insulin, Factor IX, human growth hormone, parathyroid hormone, urotensin, pituitary releasing hormones, insulin-like growth factors, erythropoietin, interleukins, antithrombin III, and other growth factors. Still other biologically active polypeptide and protein sequences are contemplated by the
- 30 invention. These peptides may be of natural or synthetic origin.

Chelators suitable for use in the present invention are those which chelate or complex calcium ions, magnesium, zinc and other cations. Preferably, the chelator is selected from the group consisting of: ethylenediaminetetraacetic acid (EDTA); ethylene glycol bis (β -aminoethyl ether) N,N,N',N' tetraacetic acid (EGTA); citrate; and 1, 4, 7, 10-tetraazacyclododecane-N,N',N'',N'''- tetraacetic acid (DOTA) DTPA (diethylenetriaminepentaacetic acid) and BAPTA (1,2 bis (2-aminophenoxy) ethane N,N,N', N' tetraacetic acid). Most preferably, the chelator is selected from EDTA or EGTA.

In order to maintain the anatomical integrity of the intestinal mucosal structure the chelator selected for use in the invention should be delivered in an amount which minimises intestinal mucosal damage. If for example EDTA or EGTA are used as the chelator, the amount of EDTA or EGTA used in the pharmaceutical composition should be in the order 0.5 to 30 grams in an adult human. Preferably, the amount of EDTA or EGTA employed in the pharmaceutical composition is in the order of 2 to 20 grams. Most preferably, the amount of EDTA or EGTA employed in the pharmaceutical composition is in the order of 4 to 15 grams. For example, the amount of EDTA or EGTA employed in the pharmaceutical composition should be between 4 and 5 grams.

Methods for preparing pharmaceutical compositions which are capable of releasing one active ingredient prior to a second active ingredient will be known to those of ordinary skill in the art. Such formulations may be prepared as liquid solutions wherein the peptides or polypeptides are encapsulated and suspended in an acid sensitive slow release capsule. Alternatively, the formulations may be prepared in solid form. For example, the peptide or protein preparation may be encapsulated in a slowly dissolving capsule which is coated with a chelating preparation that is adapted to be released upon delivery into the environment in the intestines. Alternatively, the peptide or protein preparation may be formed as microcapsules which are embedded in a rapidly dissolving chelating preparation. Other methods of formulating the composition to bring about a delayed release of a peptide or protein preparation will be known to those in the art.

The pharmaceutical composition of the present invention may also be formulated with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Examples of excipients which may be used in such a formulation include, water, saline, ethanol, dextrose glycerol, 5 pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like.

Pharmaceutical compositions prepared according to the invention may be administered in a manner compatible with the dosage formulation, and in such an amount as will be therapeutically effective. The precise amounts of active 10 ingredients required to be administered will depend to a large extent on the peptide or protein to be administered and the chelator used.

The invention also provides a method of enhancing the absorption of therapeutically effective, biologically active polypeptides or proteins so as to achieve a physiological effect in a patient requiring such therapy, wherein the 15 method comprises the step of: (i) administering to said patient in an oral formulation, an effective amount of a protein or polypeptide, and a chelating agent, said chelating agent being pre-delivered with respect to said protein or polypeptide.

Particular chelating agents suited to the method according to the invention are 20 those that chelate or complex with calcium ions, magnesium, zinc and other cations and in formulations, maintain the anatomic integrity of the intestinal mucosal lining. Of preference are EDTA and EGTA although it is also possible to use citrate and DOTA.

Preferably the pre-delivery of the chelating or complexing agent takes place 25 approximately at least 30 minutes and preferably 60 to 120 minutes prior to the delivery of the peptide or protein of interest. It may be pre-delivered up to a period of 2 hours or more, although this will vary according to the chelating agent and the peptide or protein used.

It is also preferred that the pre-deliver of the chelating agent occurs in the upper part of the small intestine, which would naturally occur when oral administration of the formulation occurs.

In a preferred embodiment of the invention there is provided a method for the treatment of diabetes, comprising the step of: orally administering to a diabetic patient an effective amount of synthetic or natural insulin in conjunction with a chelating agent such as EDTA or EGTA, the chelating agent being pre-delivered. Conveniently, the pre-delivery occurs approximately 30 minutes prior to the delivery of the peptide or protein, and may be delivered up to 2 hours prior to the active agent. Pre-delivery of the chelating agent can be made using existing enteric formulation technologies.

In particular the advantages of the invention may be summarised as follows:

1. That EDTA and other chelating agents are effective when given prior to the target protein in enhancing the absorption of proteins and other peptides from the upper small intestine. The enhancement is achieved without using bile salts or their derivatives or protease inhibitors or lipids of any kind.
2. Effective enhancement of absorption is achieved when EDTA or other chelating agents are approximately given 30 min or more prior to the target protein or peptide. Prior delivery can be readily achieved by existing formulation techniques.
3. Under the conditions described the use of EDTA and other chelating agents achieves significant enhancement of the intestinal absorption of proteins and peptides without damage to the intestinal mucosa histology.

4. Enhancement of absorption of proteins and peptides with EDTA is achieved at doses of EDTA that are known to be safe and much less than accepted toxicLD₅₀ values.

The invention will now be described with reference to laboratory trials which
5 elucidate pharmaceutical compositions prepared according to preferred embodiments of the invention. Further features of the present invention are more fully described in the following Examples. It is to be understood, however, that the following description is included solely for the purposes of exemplifying the invention, and should not be understood in any way as a restriction on the
10 broad description as set out above. In the drawings:

Figure 1 illustrates the relative plasma glucose (%) response to varying the pre-infusion times of 100mM EDTA prior to the intraduodenal administration of 200iu insulin in 100mM EDTA. Perfusion times were 120 minutes (o) *n*=11, 60 minutes (•) *n*=2, 30 minutes (Δ) *n*=2, 15 minutes
15 (+) *n*=1;

Figure 2 illustrates the relative plasma glucose (%) response to a two hour pre-infusion of varying concentrations of EDTA prior to the intraduodenal administration of 200iu insulin. Perfusion concentrations; were 10 mM EDTA (o) *n*=3, 50 mM EDTA (Δ) *n*=2, 75mM EDTA (♦) *n*=1,
20 100 mM EDTA (•) *n*=11;

Figure 3 illustrates intestinal absorption of Angiotensin II on blood pressure in rats;

Figure 4 illustrates plasma growth hormone concentrations after intestinal absorption of human growth hormone in rats;

25 **Figure 5** illustrates changes in plasma growth hormone levels after administration of growth hormone via the intestinal route;

Figure 6 illustrates the effect of insulin administration via the intestinal or intravenous route on plasma glucose content in pigs;

Figure 7 illustrates the effect of LR3-IGF-1 on plasma glucose contents after intestinal or intravenous administration of LR3-IGF-1 in pigs;

5 **Figures 8(a) to (c)** illustrates light microscope fields of a section of mucosa taken from pigs infused with EDTA;

Figure 9(a) and (b) illustrates electron micrographs of the intestines of 6 different pigs infused with EDTA;

10 **Figure 10** illustrates the effect of EDTA on plasma calcium and magnesium concentrations in pigs;

EXAMPLES

For laboratory trials, insulin, urotensin and angiotensin were selected as model therapeutic proteins because their functional effects are readily measured. This approach eliminates uncertainty about the nature of the absorbed protein with
15 respect to the preservation of its physiological functionality or its reactivation in immunoassays.

EXAMPLE 1

In control experiments 200iu of insulin was infused into the upper small intestine of rats. This had no effect on blood glucose concentrations, consistent with the
20 anticipated absence of absorption of intact functionally active insulin. In another group of rats, 200iu of insulin was infused into the intestine after a two hour pre-infusion of 10 mM EDTA (○), 50 mM EDTA (Δ), 75mM EDTA (◆) or 100 mM EDTA (●) (see figure 1). A significant decrease in blood glucose concentration was found over several hours after the infusion when compared to control rats,
25 indicating the absorption of functional insulin.

In the absence of insulin, EDTA caused the concentration of glucose in the blood to increase. By contrast, insulin delivered simultaneously with EDTA to rat models showed resultant decrease in blood glucose. An increase in the concentration of insulin in the plasma of the rat model was confirmed by radio-immunoassay of the plasma of the rats given insulin by the intestinal route when EDTA was infused into the intestine at concentrations as low as 50 millimolar, but not when this was done in the absence of EDTA (Figure 1).

EXAMPLE 2

Example 1 was repeated except the dose of insulin given by the intestinal route was varied between 12.5 international units to 200 international units. The effect on the blood glucose was then measured. The effect was found to be proportional to the amount of insulin delivered. EDTA was effective in enhancing absorption of insulin when present in concentrations from 50 to 150 millimolar in an infused solution. The enhancing effect was also seen when the intestine was exposed to EDTA solution for 0.5 to 2 hours before the insulin was presented (Figure 2).

EXAMPLE 3

Rats were prepared with upper intestinal fistulas and the carotid artery was cannulated for sampling of blood and measurement of blood pressure. In this experiment urotensin I was delivered into the intestine together with a 74 milligram solution of EDTA. When 50µg/100g body weight of urotensin I was infused alone into the intestine of control rats, there was minimal change in blood pressure of the subject animals. However after infusion of 50µg/100g body weight urotensin I following pre-infusion for 2 hours of EDTA, the blood pressure decreased by 70 mmHg indicating that intact urotensin I was absorbed from the intestinal tract in the presence of EDTA. The blood pressure was significantly decreased compared with control rats confirming the absorption of functionally active urotensin I. There was a dose-response relationship between the decrease in blood pressure and the infused dose of urotensin I. A similar

effect was found with urotensin II which was also effective in decreasing the blood pressure of the subject rats. In other studies in rats the intestinal absorption of parathyroid hormone and corticotrophin-releasing hormone were also achieved by prior infusion of EDTA but not without pre-infusion of a
5 chelator.

EXAMPLE 4

Rats were prepared with upper intestinal fistulas and the carotid artery was cannulated for sampling of blood and measurement of blood pressure. In this trial 100 μ g of angiotensin II was delivered into the intestine together with a 74
10 milligram solution of EDTA. When 100 μ g of angiotensin II was infused alone into the intestine of control rats, there was no change in blood pressure of the subject animals. However, after infusion of angiotensin after pre-infusion for 2 hours of EDTA, the blood pressure increased indicating that intact angiotensin was absorbed from the intestinal tract in the presence of EDTA. The blood
15 pressure was significantly increased compared with control rats confirming the absorption of functionally active angiotensin II. Larger doses of angiotensin II up to 500 μ g caused greater increments of blood pressure. A second dose of angiotensin II (100 μ g) given 2 hours after the first was also effective in raising the blood pressure of the subject rats.

20 Similar trials conducted with EGTA proved as effective as EDTA. Histological examination of samples taken from the small intestine of the rats infused with chelators showed no damage to the mucosa.

In a group of rats given daily for three weeks by gavage a dose of EDTA double that effective in enhancing absorption of peptides the rats showed no untoward
25 effects and the treated group showed growth rates comparable to a control group of rats gavaged with a saline solution.

EXAMPLE 5

Angiotensin II is an 8 amino acid polypeptide that acts as a potent vasoconstrictor and raised blood pressure. The absorption of Angiotensin II was shown to be enhance in rats infused with EDTA, as shown by the consequent
5 increase in blood pressure (Figure 3). In control studies rats given Angiotensin II in the absence of EDTA, there was no change in blood pressure.

EXAMPLE 6

Human Growth Hormone (22,000 daltons) at a dose of 1 mg/kg was also shown to be absorbed by the intestine when rats were pre-infused for 2 hours with
10 EDTA. Growth Hormone (GH) was detected by immunoassay in plasma within 10 min of injection into the intestine, as shown in Figure 4. In control rats not treated with EDTA no GH activity was detected when GH was injected into the intestine.

EXAMPLE 7

15 To extend the understanding of the applicability of the invention, trials were conducted on pigs. In pigs the experimental design was similar to procedures in the rat, with a cannula placed in the jugular vein for blood sampling and another placed in the duodenum for the pre-infusion for 2 hours of 100mM EDTA and administration of proteins. When 0.2 mg/kg GH was injected into pigs via the
20 duodenum after pre-infusion with a 1.1 gram solution of EDTA, the hormone was detected by immunoassay in plasma at 10 min and was detectable over the following 2 hr (Figure 5). The absorption of GH was estimated to be between 1-3% of the dose by comparison with intravenous injection of the hormone (Figure 5). In the absence of pre-infusion of EDTA no absorbed GH was detectable.
25 Similar results were found when pigs were infused with porcine somatotrophin which was absorbed after pre-infusion with EDTA but not otherwise.

In pigs the gastrointestinal absorption of insulin was also enhanced by EDTA, shown by the decrease in the concentration of plasma glucose (Figure 6). Moreover in the presence of EDTA the absorption of the insulin-like growth factor LR³IGF-1 was also enhanced, as measured by its glucose lowering effect (Figure 7). When given without pre-infusion of EDTA neither insulin nor LR³IGF-1 decreased with plasma glucose concentration.

Compared with rats the studies in pigs showed that a significantly smaller dose of insulin was needed to achieve a sustained decrease in blood glucose. The absorption of insulin was approximately 10% and of LR³IGF-1 approximately 5% by comparison with responses after intravenous injection of the respective hormones. However, direct comparisons of blood glucose responses following intravenous or intestinally administered insulin are difficult since insulin absorbed from the intestine enters the portal vein and a large proportion is extracted by the liver on the first pass (Tranberg, K-G. (1979) "Hepatic uptake of insulin in man." Am. J. Physiol. 237:E509-E518). In contrast insulin given into a systemic vein is distributed to many tissues in the body, with only a minority travelling to the liver in a first pass. Consequently, the physiological responses, such as movement of glucose from plasma to tissues, will differ between the two modes of delivery. Under normal physiological circumstances insulin secreted from the Islets of Langerhans in the pancreas is released into the portal vein, so in this respect absorption from the intestine can be said to mimic more closely the normal physiological route of delivery.

Biopsies of small intestinal mucosa from pigs infused with EDTA were examined by light and electron microscopy. Figure 8 is a light microscope field of a section of mucosa taken from pigs infused with EDTA. This composite figure shows sections from 5 different pigs and shows the lack of damage to the intestine. Figure 9 shows electron micrographs of the intestines of 6 different pigs infused with EDTA. The intracellular organelles were intact, there was no damage to the microvilli and junctions between intestinal cells were similar to sections from control animals not treated with EDTA.

Measurements were made in pigs to evaluate the effect of EDTA on plasma calcium and magnesium concentrations. As shown in Figure 10, there was no effect of EDTA on plasma calcium or magnesium levels.

The results from these experiments in rats and pigs provide evidence of enhancement of the gastrointestinal absorption of intact proteins. The absorption of insulin was estimated to be approximately 10% of the dose although this may be an underestimate because of the rapid catabolism of insulin delivered by the portal route compared with the systemic intravenous route. The level of absorption achieved was much higher than in the absence of the pre-infusion of chelator, when only approximately 0.1% was absorbed.

It will be appreciated that the oral absorption of the functionally active peptide or polypeptide as demonstrated by this invention will enable the benefits of orally administrable drugs to be extended to the delivery of peptidic sequences. It will also be appreciated that the scope of this invention extends to chelating and complexing agents and peptides and polypeptides, both natural and synthetic other than those specifically contemplated herein. In a preferred application in humans the subject will take an oral preparation of a chelator such as EDTA in liquid or solid form accompanied by a peptide preparation in a formulation such that its passage from the stomach into the upper intestinal tract will occur some 30 minutes or longer later than the passage of the chelator, to achieve the essential feature of prior delivery of the chelator so that absorption of the peptide is enhanced. Such formulation of the peptide is accomplished by a particular coating to prevent digestion in the stomach and to delay passage into the upper small intestine, techniques which are known in pharmaceutical science.

The CLAIMS defining the invention are:

1. A pharmaceutical composition for the delivery of proteins and/or polypeptides, comprising: (i) one or more therapeutically effective peptides or polypeptides; and (ii) a chelating agent, wherein said composition is adapted to pre-deliver the chelating agent.
5
2. A pharmaceutical composition according to claim 1 wherein the composition is adapted to release the chelator upon entry into the intestinal tract or soon thereafter, then after a suitable time period release the peptide or protein.
- 10 3. A pharmaceutical composition according to claim 1 wherein the delay between the release of the chelator and the protein or peptide is greater than about 30 minutes.
4. A pharmaceutical composition according to claim 1 wherein the chelator is released 60 to 120 minutes prior to the release of the peptide or protein of interest.
15
5. A pharmaceutical composition according to claim 1 wherein the chelator is pre-delivered at least 2 hours before the peptide or protein.
6. A pharmaceutical composition according to claim 1 wherein the peptide or polypeptide preparation is selected from the group consisting of: Insulin, Factor IX, human growth hormone, parathyroid hormone, urotensin, pituitary releasing hormones, insulin-like growth factors, erythropoietin, interleukins, antithrombin III, or other growth factors.
20
7. A pharmaceutical composition according to claim 1 wherein the chelator selected for use in the present invention is capable of complexing at least calcium ions.
25

8. A pharmaceutical composition according to claim 1 wherein the chelator selected for use in the present invention is capable of complexing at least magnesium.
9. A pharmaceutical composition according to claim 1 wherein the chelator selected for use in the present invention is capable of complexing at least zinc.
10. A pharmaceutical composition according to claim 1 wherein the chelator is selected from the group consisting of: ethylenediaminetetraacetic acid; ethylene glycol bis (β -aminoethyl ether) N,N,N',N' tetraacetic acid; citrate; and 1, 4, 7, 10-tetraazacyclododecane-N,N',N'',N'''- tetraacetic acid DTPA (diethylenetriaminepentaacetic acid) and BAPTA (1,2 bis (2-aminophenoxy) ethane N,N,N', N' tetraacetic acid).
11. A pharmaceutical composition according to claim 1 wherein the chelator is ethylenediaminetetraacetic acid.
12. A pharmaceutical composition according to claim 1 wherein the chelator is ethylene glycol bis (β -aminoethyl ether) N,N,N',N' tetraacetic acid.
13. A pharmaceutical composition according to claim 1 wherein the chelator is administered in an amount which maintains the anatomical integrity of the intestinal mucosal structure
14. A pharmaceutical composition according to claim 1 wherein the chelator is administered in an amount of about 0.5 to 30 grams.
15. A pharmaceutical composition according to claim 1 wherein the chelator is administered in an amount of about 2 to 20 grams.
16. A pharmaceutical composition according to claim 1 wherein the chelator is administered in an amount of about 10-15 grams.

17. A method of enhancing the absorption of therapeutically effective, biologically active polypeptides or proteins so as to achieve a physiological effect in a patient requiring such therapy, wherein the method comprises the step of: (i) administering to said patient in an oral formulation, an effective amount of a protein or polypeptide, and a chelating agent, said chelating agent being pre-delivered with respect to said protein or polypeptide.
18. A method according to claim 17 wherein the chelating agent is delivered to the upper part of the small intestine.
19. A method according to claim 17 wherein the composition is adapted to release the chelator upon entry into the intestinal tract or soon thereafter, then after a suitable time period release the peptide or protein.
20. A method according to claim 17 wherein the delay between the release of the chelator and the protein or peptide is greater than about 30 minutes.
21. A method according to claim 17 wherein the chelator is released 60 to 120 minutes prior to the release of the peptide or protein of interest.
22. A method according to claim 17 wherein the chelator is pre-delivered at least 2 hours before the peptide or protein.
23. A method according to claim 17 wherein the peptide or polypeptide preparation is selected from the group consisting of: insulin, Factor IX, human growth hormone, parathyroid hormone, urotensin, pituitary releasing hormones, insulin-like growth factors, erythropoietin, interleukins, antithrombin III, or other growth factors.
24. A method according to claim 17 wherein the chelator selected for use in the present invention is capable of complexing at least calcium ions.

25. A method according to claim 17 wherein the chelator selected for use in the present invention is capable of complexing at least magnesium.
26. A method according to claim 17 wherein the chelator selected for use in the present invention is capable of complexing at least zinc.
- 5 27. A method according to claim 17 wherein the chelator is selected from the group consisting of: ethylenediaminetetraacetic acid; ethylene glycol bis (β -aminoethyl ether) N,N,N',N' tetraacetic acid; citrate; and 1, 4, 7, 10-tetraazacyclododecane-N,N',N'',N'''- tetraacetic acid DTPA (diethylenetriaminepentaacetic acid) and BAPTA (1,2 bis (2-aminophenoxy) ethane N,N,N', N' tetraacetic acid).
- 10 28. A method according to claim 17 wherein the chelator is ethylenediaminetetraacetic acid.
29. A method according to claim 17 wherein the chelator is ethylene glycol bis (β -aminoethyl ether) N,N,N',N' tetraacetic acid.
- 15 30. A method according to claim 17 wherein the chelator is administered in an amount which maintains the anatomical integrity of the intestinal mucosal structure
31. A method according to claim 17 wherein the chelator is administered in an amount of about 0.5 to 30 grams.
- 20 32. A method according to claim 17 wherein the chelator is administered in an amount of about 2 to 20 grams.
33. A method according to claim 17 wherein the chelator is administered in an amount of about 10-15 grams.

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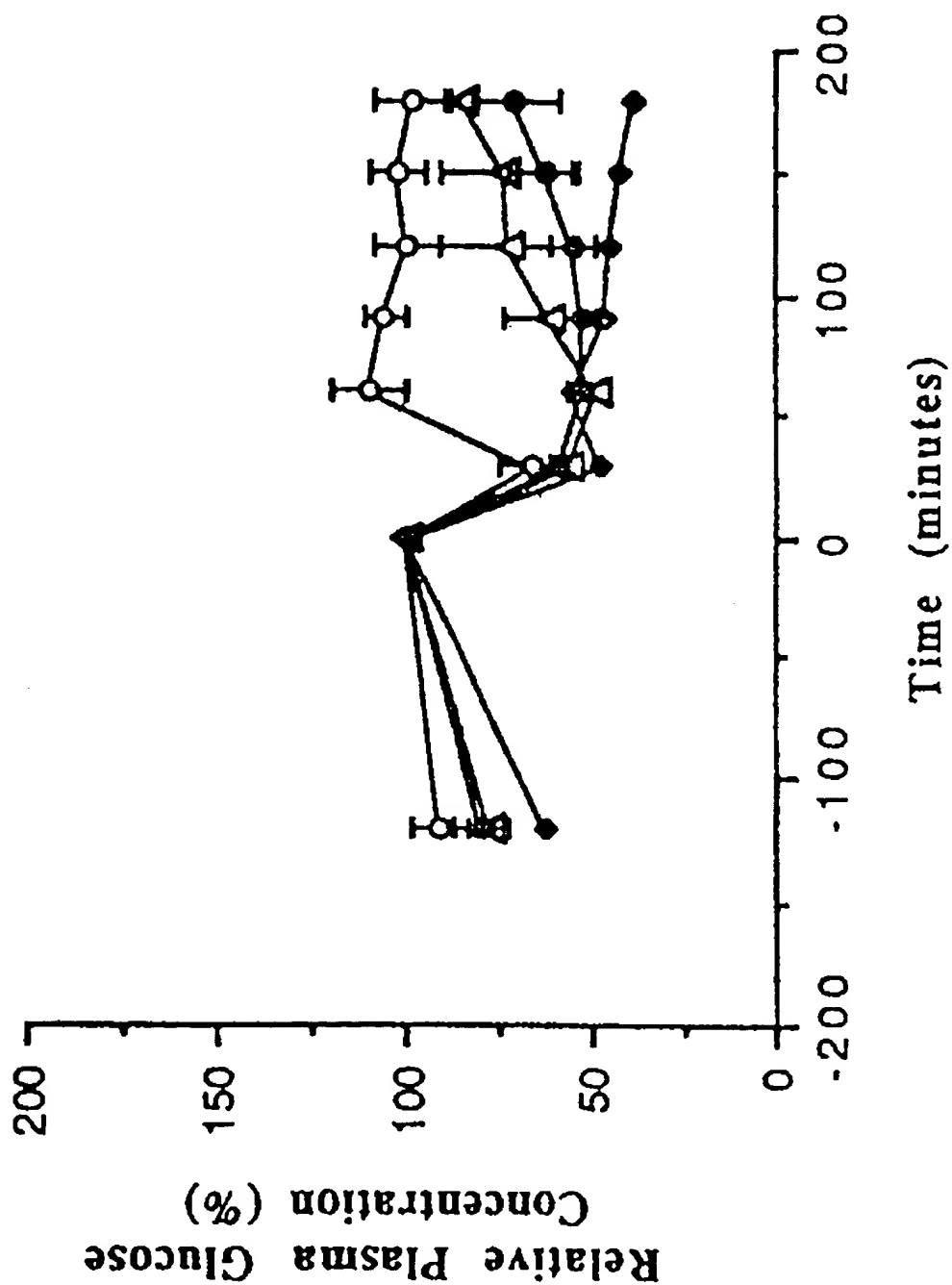


Fig 1

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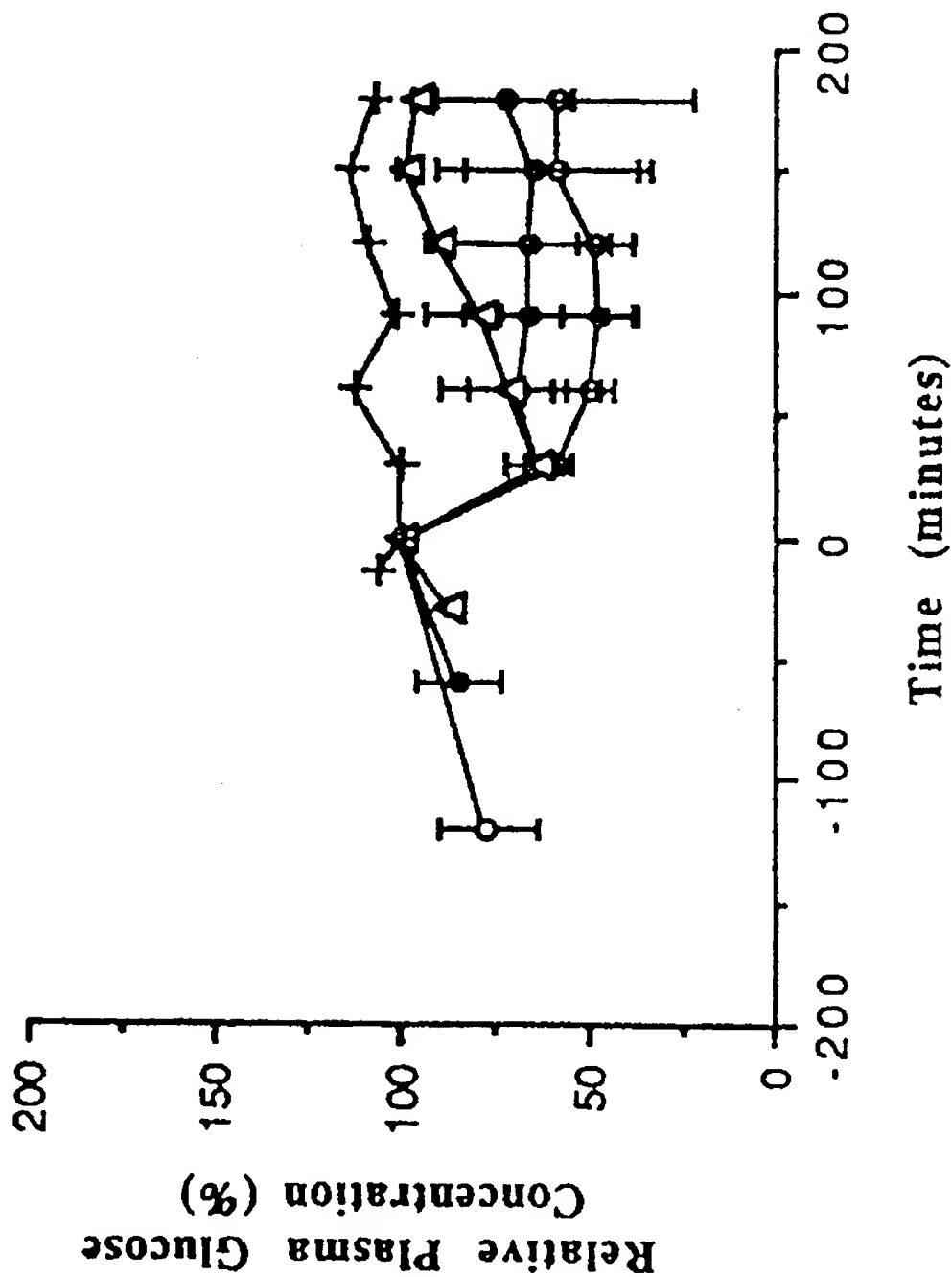
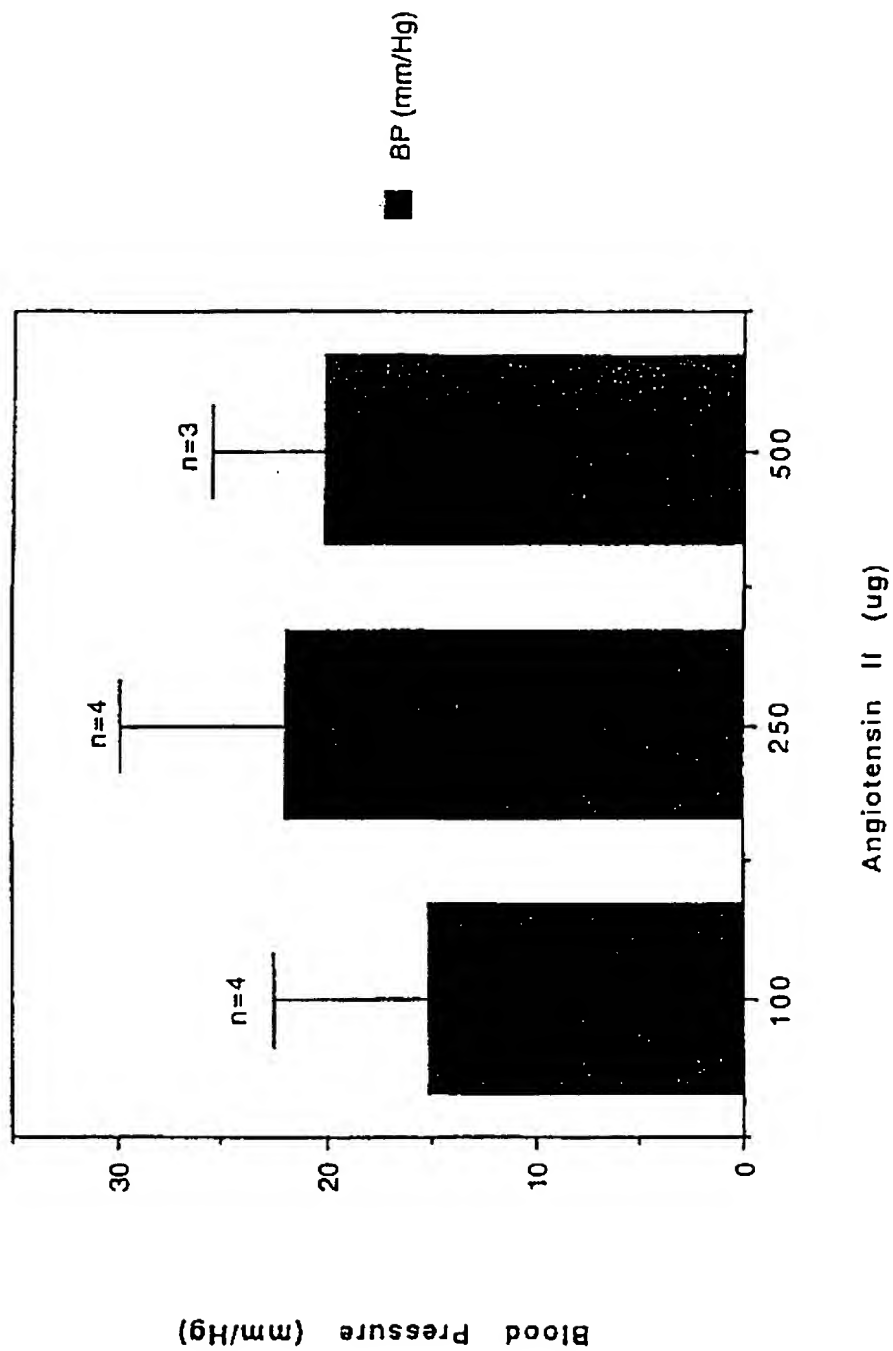


Fig 2

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**Fig. 3**

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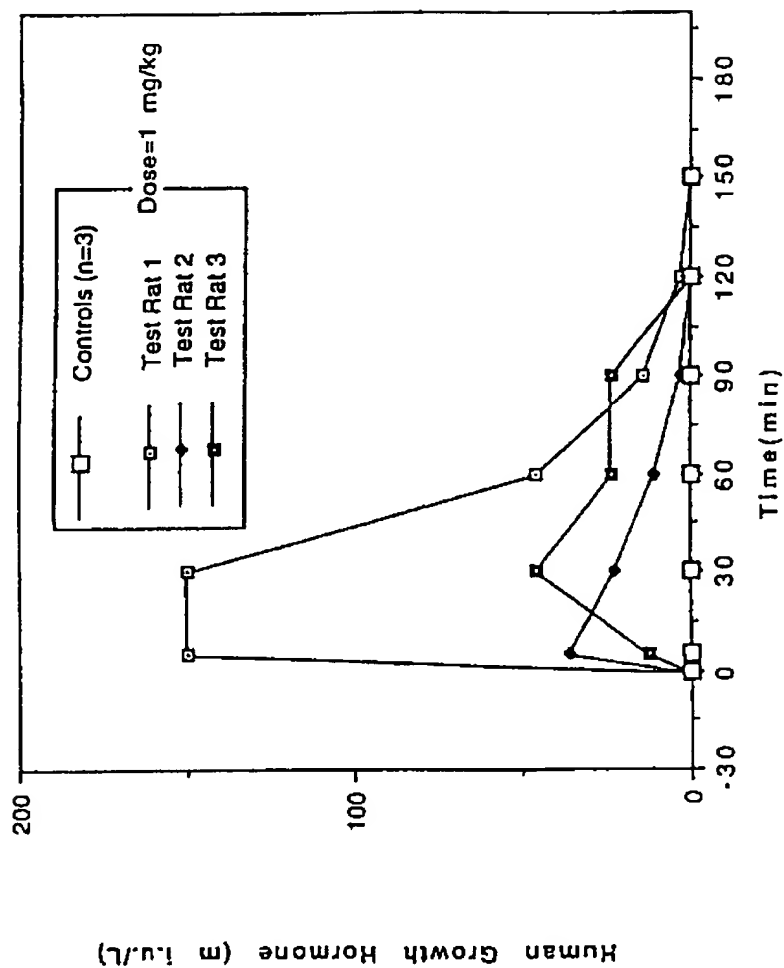
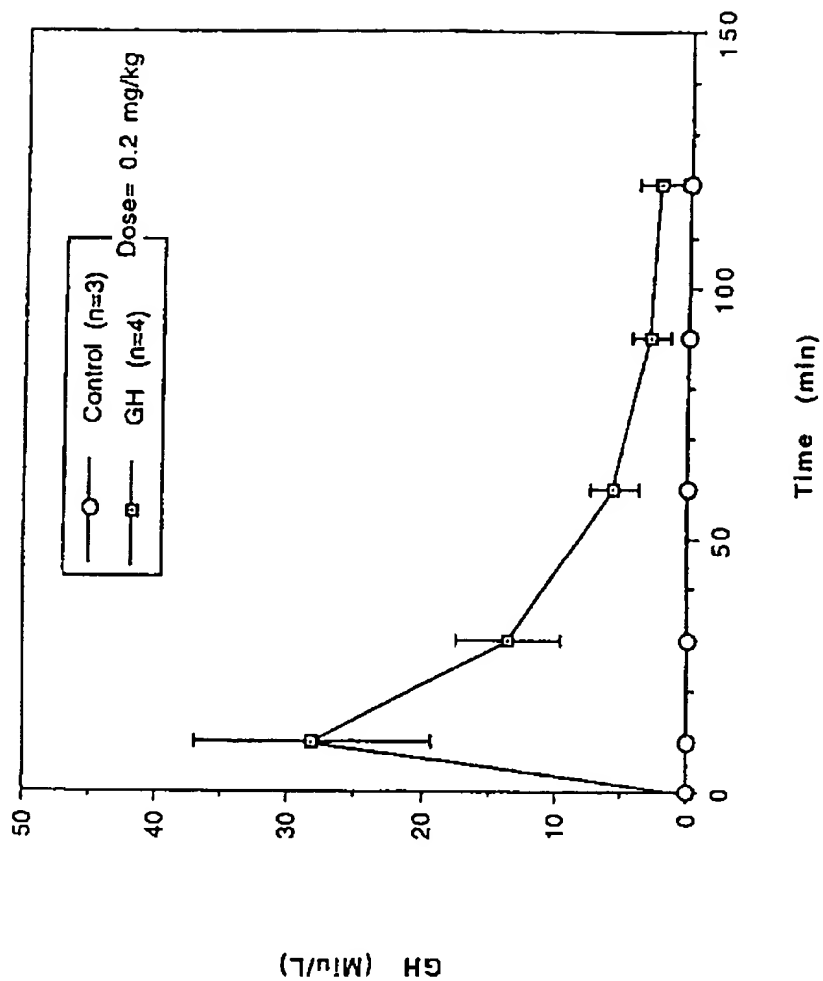
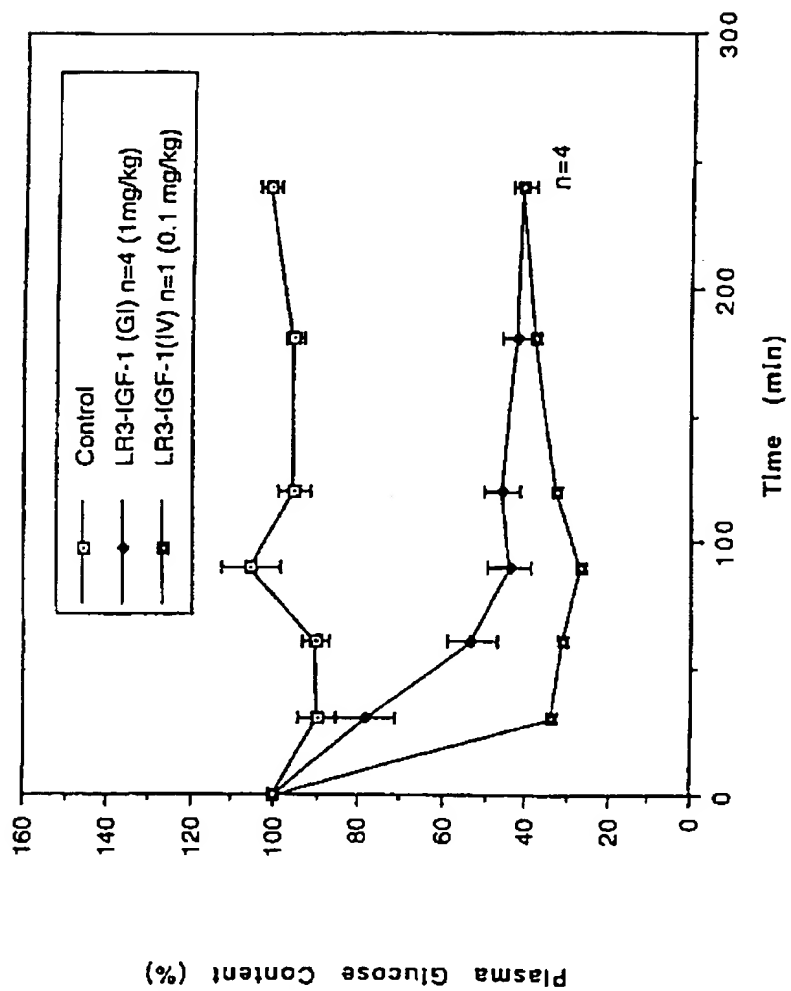


Fig. 4.

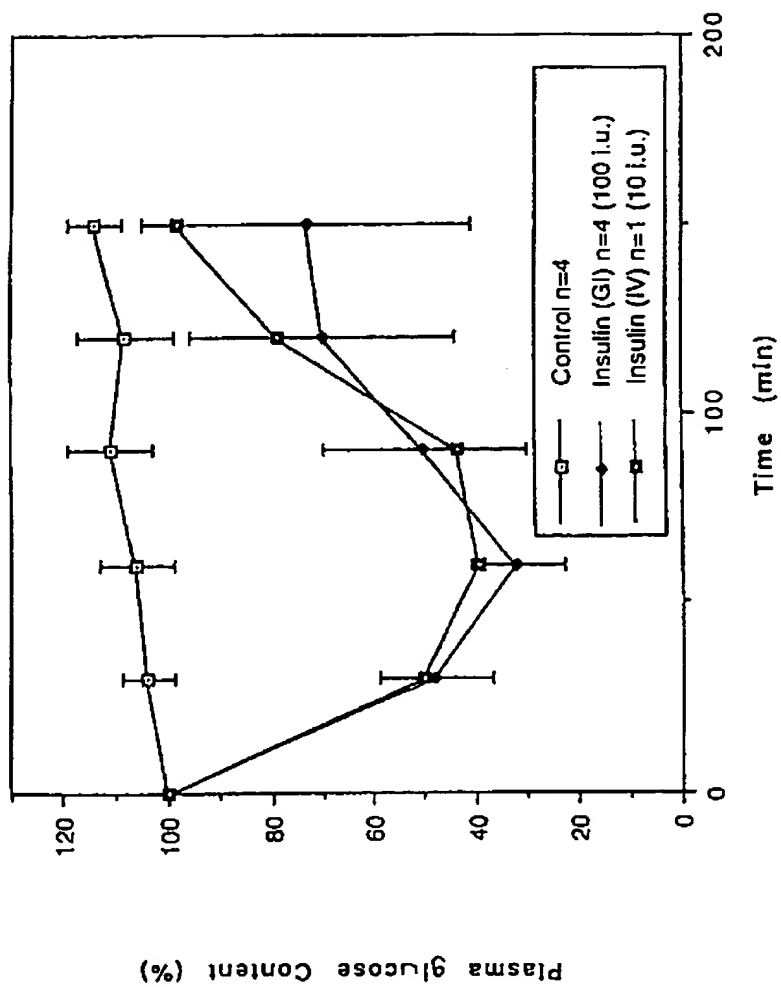
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**Fig. 5.**

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**Fig. 6.**

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**Fig. 7.**

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Fig. 8 A

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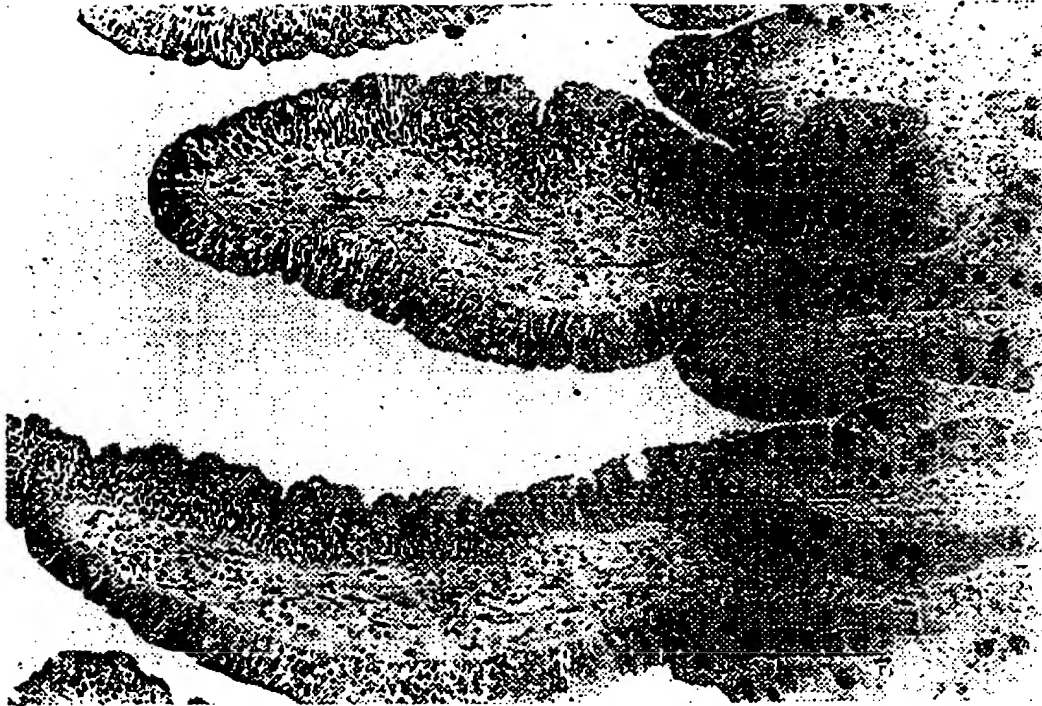


Fig. 8B

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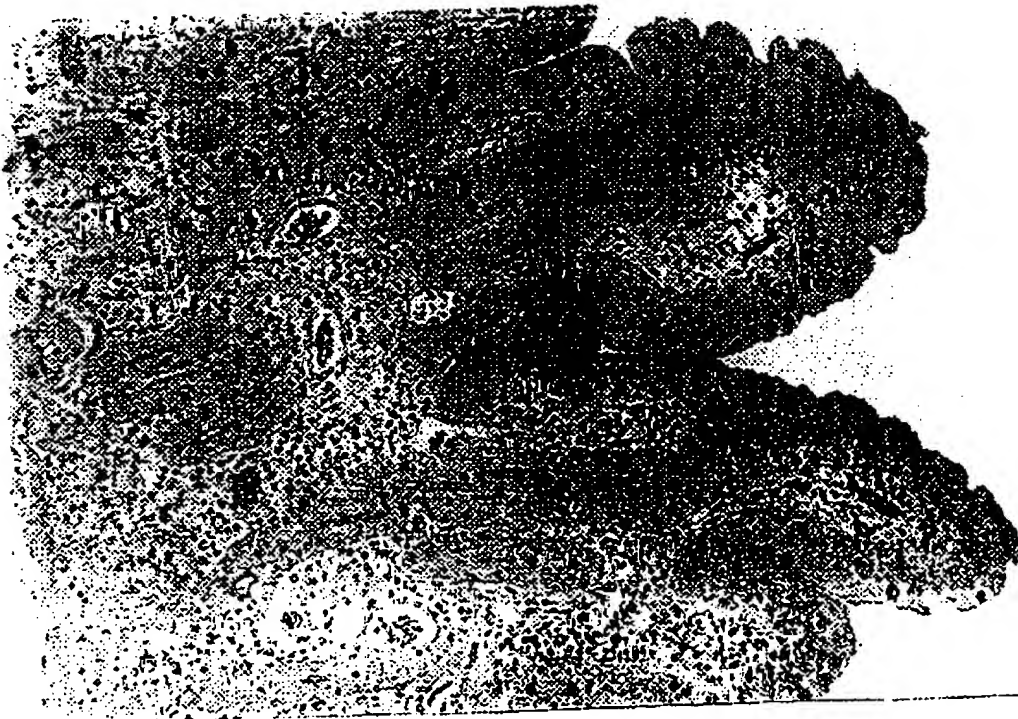
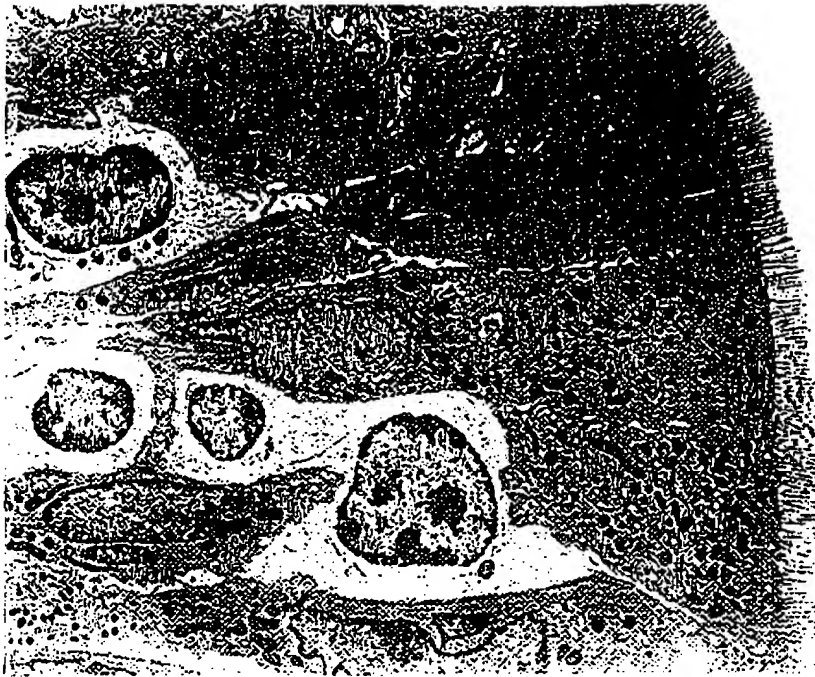


Fig. 8 c.

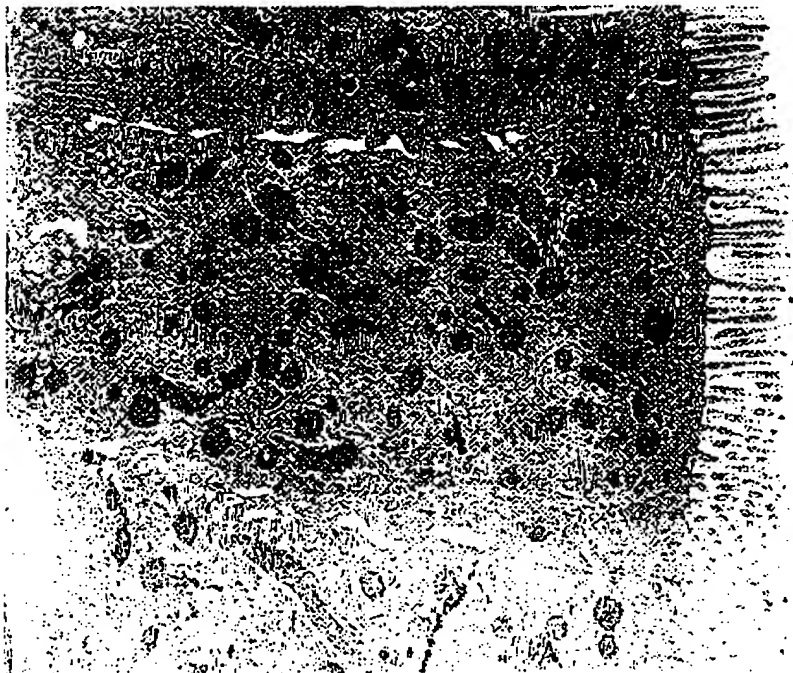
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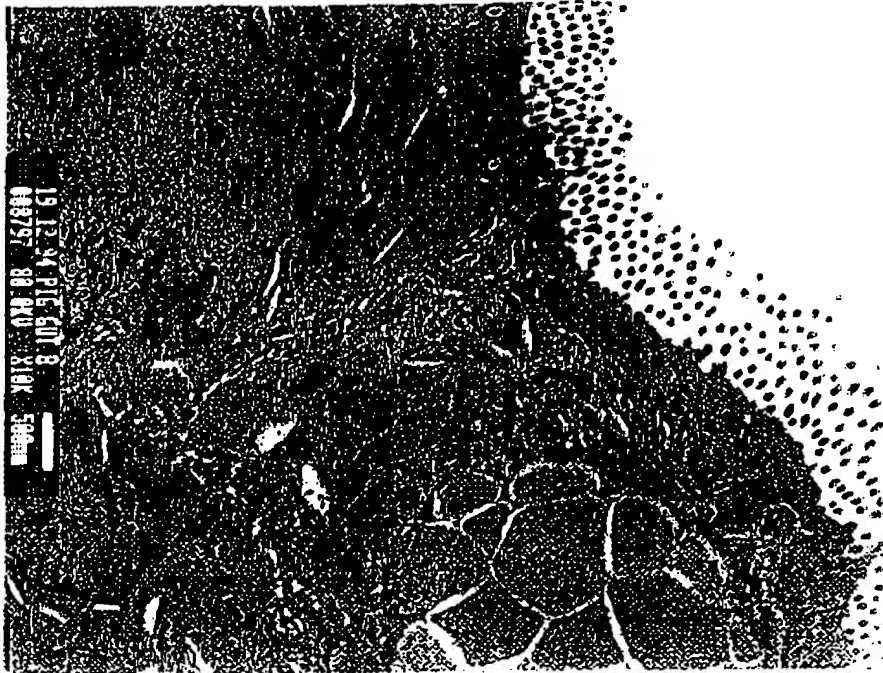
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FIG. 9A

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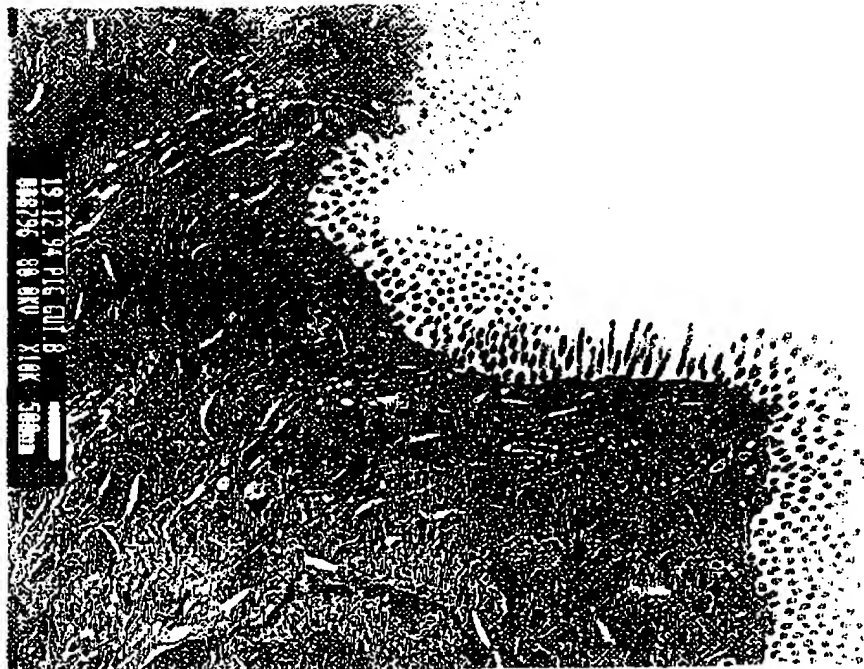
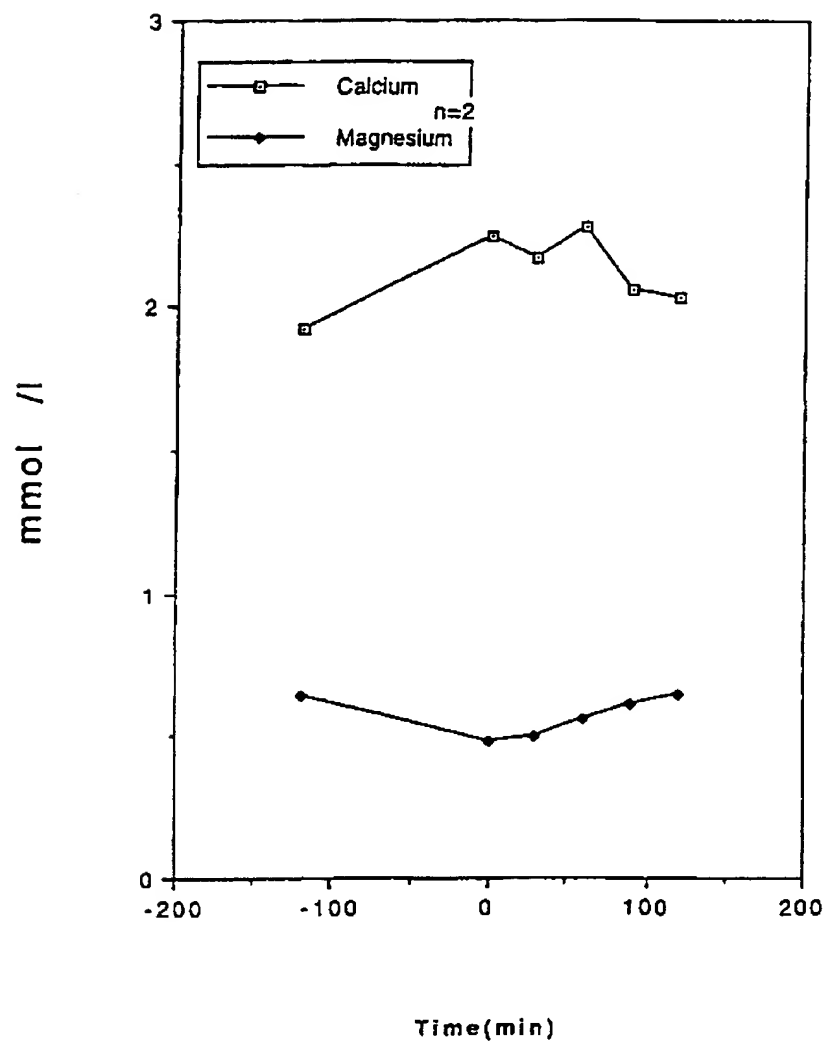


Fig. 9 B.

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**Fig. 10**

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00344

A. CLASSIFICATION OF SUBJECT MATTER		
Int Cl ⁶ : A61K 9/22, 37/26, 37/36, 37/43, 37/64, 47/12		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) IPC A61K 9/22, 37/26, 37/36, 37/43, 37/64, 47/12		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched AU:IPC as above		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) DERWENT and CHEMICAL ABSTRACTS: Keywords - (CHELAT: or EDTA or EGTA or DOTA or DTPA or BAPTA) and (PEPTIDE: or POLYPEPTIDE: or PROTEIN: or INSULIN or FACTOR(IX or GROWTH(HORMONE: or PARATHYROID(HORMONE or UROTENSIN or PITUITARY(RELEASING(HORMONE or INSULIN(LIKE(GROWTH(FACTOR or ERYTHROPOIETIN or INTERLEUKIN: or ANTITHROMBIN or GROWTH(FACTOR:)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 88/02635 A (CYTOGEN CORP), 21 April 1988	1-16
X	US 5182258 A (ORBON CORP), 26 January 1993	1-16
X	WO 89/09610 A (CETUS CORP), 19 October 1989	1-16
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex		
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Date of the actual completion of the international search 20 August 1997		Date of mailing of the international search report 28 AUG 1997
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929		Authorized officer HELLENE FLAME-HIOTAKIS Telephone No.: (02) 6283 2103

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00344

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Derwent Abstract Accession No. 94-329954/41, Class A96 B05 (B07) JP 06256219 A (HISAMITSU PHARM CO. LTD), 13 September 1994	1-16
X	US 5283236 A (ORBON CORP), 1 February 1994	1-16
P,X	EP 727221 A (KOREA RESEARCH INSTITUTE OF CHEMICAL TECHNOLOGY), 21 August 1996	1-16
X	AU 50602/93 A (HYBRITECH INC), 26 May 1994	1-16
X	WO 93/18797 A (MALLINCKRODT MEDICAL INC), 30 September 1993	1-16
X	WO 93/04702 A (MALLINCKRODT MEDICAL INC) 18 March 1993	1-16
X	WO 92/08494 A (STERLING WINTHROP INC), 29 May 1992	1-16
X	WO 91/15509 A (CALIFORNIA BIOTECHNOLOGY INC), 17 October 1991	1-16
X	Derwent Abstract Accession No. 89-071651/10 class B07 (B05), JP 01022817 A (TERUMO CORP), 25 January 1989	1-16
P,X	WO 96/40073 A (ALKERMES CONTROLLED THERAPEUTICS INC), 19 December 1996	1-16
X	WO 96/15816 A (AMGEN INC. USA), 30 May 1996	1-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/AU 97/00344

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Patent Document Cited in Search Report				Patent Family Member			
WO	88/02635	AU	81528/87	ZA	8707727		
WO	89/09610	AU	34473/89	EP	423244	ES	2013667
		US	5215743				
AU	50602/93	CA	2102848	EP	623675	JP	6/343489
		ZA	9308243				
WO	93/18797	AU	39675/93	EP	636032		
WO	93/04702	AU	24625/92	CA	2113995	EP	600992
		US	5384113				
WO	92/08494	AU	90291/91	EP	624097	FI	932083
		NO	931631	NZ	240521	US	5367080
WO	91/15509	AU	76925/91	CA	2079780	EP	527781
		US	5217954				
WO	96/40073	AU	59724/96				
WO	96/15816	AU	43667/96				
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